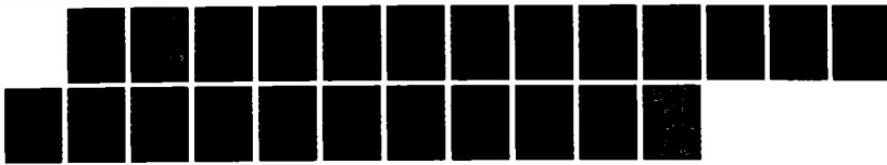


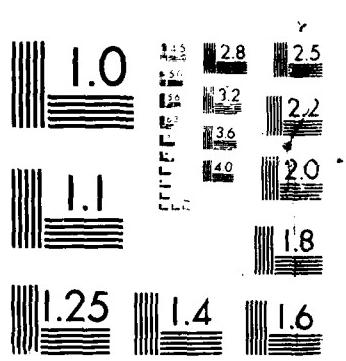
AD-A195 177 SAFETY TESTING OF DENGUE-2 AND DENGUE-4 VIRUSES FOR USE 1/1
IN HUMAN CHALLENGE EXPERIMENTS (U) JOHN A BURNS SCHOOL
OF MEDICINE HONOLULU HI N J MARCHETTE 01 APR 88

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Safety Testing of Dengue-2 and Dengue-4 Viruses
for Use in Human Challenge Experiments

Final Report

Nyven J. Marchette

April 1, 1988

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-83-C-3047

University of Hawaii
School of Medicine
Honolulu, Hawaii 96816

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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.



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Safety testing of attenuated dengue virus vaccines and challenge viruses:

Dengue 2 (PR-159) Parent, non-attenuated; January 1982.
Lot No. 1 (Human challenge virus).

Dengue 4 (H-241) Parent, non-attenuated; December 1982.
Lot No. 1 (Human challenge virus).

Dengue 3 (H53489) Production seed and control fluids

For each of the viruses, the following substances were tested separately for sterility and presence of adventitious agents:

Freeze dried virus;
Virus fluids, unclarified;
Control fluids, day 7, unclarified;
Control fluids, day 14, unclarified;
Pre-inoculation fluids.

In addition, the identify of each virus was determined by breakthrough neutralization tests.

All tests were concluded satisfactorily. No adventitious agents were detected and the identity of the viruses was confirmed. The detailed final report for each virus tested is appended.

Initially, there were problems with the Dengue 4 challenge virus in that carbon dioxide from the dry ice in the shipping container leaked into the vials and inactivated the virus. This was eventually corrected and the third lot of this virus arrived in good shape and the safety testing completed.

No equipment was purchased on this contract and there is no government property remaining.

Department of Tropical Medicine & Medical Microbiology
 John A. Burns School of Medicine
 University of Hawaii
 FINAL REPORT

Test item: Dengue 2 (PR-159) parent, non-attenuated, Jan 1982, Lot no. 1 (human challenge virus)

A. Sterility Test*

1) Microbial Sterility: Pre-inoculation fluid

<u>Test Volume</u>	<u>Temp</u>	<u>Media</u>	<u>Inoc. size/ Culture</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
5 ml	Room	TSB (BBL, Cokessville, Md)	1 ml/40 ml	7 Feb 83	21 Feb 83	Satisfactory	Book 45 pp 2-5
5 ml	31°C	Thio.(BBL, Cokessville, Md)	1 ml/40 ml	7 Feb 83	21 Feb 83	Satisfactory	Book 45 pp 2-5

2) Microbial Sterility: Control fluid

<u>Test Volume</u>	<u>Temp</u>	<u>Media</u>	<u>Inoc. size/ Culture</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
10 ml	Room	TSB (BBL, Cokessville, Md)	1 ml/40 ml	7 Feb 83	21 Feb 83	Satisfactory	Book 45 pp 2-5
10 ml	31°C	Thio.(BBL, Cokessville, Md)	1 ml/40 ml	7 Feb 83	21 Feb 83	Satisfactory	Book 45 pp 2-5
6 ml		Mycoplasma broth and Agar 1 ml/tube x 4 tubes 0.2 ml/plate x 10 plates (BBL, Cokessville, Md)		7 Feb 83	8 Mar 83	Satisfactory	Book 45 pp 2-5

3) Microbial Sterility: Candidate Dengue Virus, Unclarified Fluid Pool

<u>Test Volume</u>	<u>Temp</u>	<u>Media</u>	<u>Inoc. size/ Culture</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
10 ml	Room	TSB (BBL, Cokessville, Md)	1 ml/40 ml	18 Apr 83	25 Apr 83	Satisfactory	Book 45 pp 10-12
10 ml	31°C	Thio.(BBL, Cokessville, Md)	1 ml/40 ml	18 Apr 83	25 Apr 83	Satisfactory	Book 45 pp 10-12
6 ml		Mycoplasma broth and Agar 1 ml/tube x 4 tubes 0.2 ml/plate x 10 plates (BBL, Cokessville, Md)		18 Apr 83	16 May 83	Satisfactory	Book 45 pp 10-12

* Media will show no growth at 18-24 hours incubation for sterility.

TSB: Will support growth of Bacteroides vulgaris ATCC 8482 and Micrococcus lutea ATCC 12-9341.

THIC: Will support growth of Micrococcus lutea ATCC 12-9341 and Candida albicans ATCC E-10231.

Mycoplasma media: Will support growth of M. pneumoniae ATCC 15531 and N. arthritidis

Den-2 (PR-159) human challenge virus

B. Cell Culture Safety Test for Candidate Virus, Fluid Pool

Pre-inoculation fluid: Inoculation size--3.5 ml/25 cm² flask

<u>Cells</u>	<u>No. of Culture</u>	<u>Volume Tested</u>	<u>Days Observed</u>	<u>Results</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Data Location</u>
Primary green monkey kidney. Source--T3M Lot # IX	3 flasks	10 ml	14	Satisfactory	13 Apr 83	27 Apr 83	Book 44 pp 16-20
Subculture PGMK cell Source--T3N Lot # IX	3 flasks	10 ml	14	Satisfactory	27 Apr 83	11 May 83	Book 44 pp 22-23
Primary Rhesus monkey kidney. Source--Flow Lot # 56555	3 flasks	10 ml	14	Satisfactory	13 May 83	27 May 83	Book 44 pp 53-54
Primary rabbit kidney. Source--Flow Lot # 56210	3 flasks	10 ml	14	Satisfactory	13 Apr 83	27 Apr 83	Book 44 pp 16-18
WI-38 cells, Passage--31 Source--T3M	3 flasks	10 ml	14	Satisfactory	13 Apr 83	27 Apr 83	Book 44 pp 16-18

Den-2 (PR-159) human challenge virus

3. Cell Cultures (cont.)

Candidate Dengue Virus, Fluid Pool

- 1) Infectivity Titer: (Mean of 3 determinations) 2.1×10^4 pfu/ml
- 2) Safety Test: Neut. virus inoc.: 1.7 ml per 25 cm² flask. Control fluid inoc.: 3.5 ml per 25 cm² flask
Unneut. virus inoc.: 3.5 ml per 25 cm² flask

<u>Cell#</u>	<u>Neut. Virus Vol.</u>	<u>No. of Culture</u>	<u>Con. Fluids Vol.</u>	<u>No. of Culture</u>	<u>Days Observed</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
Primary Green monkey kidney Source--T3M Lot # IX	5 ml	3 flasks	10 ml	3 flasks	14	13 Apr 83	27 Apr 83	Satisfactory	Book 44 pp 16-20
Subculture PGMK Source--T3M Lot # VIII, IX	10 ml	3 flasks	10 ml	3 flasks	14	27 Apr 83 27 May 83	11 May 83 10 Jun 83	Satisfactory	Book 44 pp 22-23 Book 44 pp 59-61
Primary Rhesus monkey kidney Source--Flow Lot # 56555, 57083	5 ml	3 flasks	10 ml	3 flasks	14	13 May 83 11 Jul 83	27 May 83 25 Jul 83	Satisfactory	Book 44 pp 53-54 Book 44 p 78
Primary rabbit kidney Source--Flow Lot # 56597	5 ml	3 flasks	10 ml	3 flasks	14	13 Apr 83 11 May 83	27 Apr 83 25 May 83	Satisfactory	Book 44 pp 16-18 Book 44 pp 50-52
WI-38 passage 30, 31 Source--T3M	5 ml	3 flasks	10 ml	3 flasks	14	13 Apr 83 19 May 83 9 Jun 83	27 Apr 83 2 Jun 83 23 Jun 83	Satisfactory	Book 44 pp 16-18 Book 44 pp 55-56 Book 44 p 70

Den-2. (PR-159) human challenge virus

C. Animal inoculation

Animal	No. inoculated			Date Initiated	Date Completed	Results	Data Location
	Neut. Virus	Unneut. Virus	Con. Fluids				
Adult mice, female, Swiss Webster, 17-20 g* 0.03 ml i.c. + 0.5 ml i.p.	---	20	---	21 Jan 83	11 Feb 83	Satisfactory	Book 44 pp 14-15
Suckling mice, Swiss Webster, 1 day* 0.01 ml i.c. + 0.1 ml i.p.	8 litters	--	3 litters	20 Feb 83	13 Mar 83	Satisfactory	Book 44 pp 9-13
Subpassage (suckling mice) 0.01 ml i.c. + 0.1 ml i.p.	4 litters	--	--	21 Mar 83	4 Apr 83	Satisfactory	Book 44 pp 27-29
Guinea pig, male, Hartley, 200-250 g* 0.1 ml i.c. + 5 ml i.p.	----	2	2	21 Jan 83	4 Mar 83	Satisfactory	Book 44 pp 3-6
Rabbit, male, New Zealand, 1.4-i.9 kg* 9 ml s.c. + 0.25 ml x 4 i.d.	----	2	2	21 Jan 83	11 Feb. 83	Satisfactory	Book 44 pp 3-8

i.c. = intracerebral
 i.p. = intraperitoneal
 s.c. = subcutaneous
 i.d. = intradermal

Procedure for identification:

Mouse Safety Test: Each individual cage is labeled with the number, virus lot number and date of test.

Guinea Pig Safety Test: Guinea Pig ears are tattooed with individual serial numbers. Each cage is labeled with the serial number, virus lot number and date of test.

Rabbit Safety Test: Rabbit ears are tattooed with an individual serial number. Cage is labeled to show virus lot number and date of test.

* Source of mice and guinea pigs: Simonson Labs, Gilroy, California.

Source of rabbits: Smitty's Farm, Kaneohe, Hawaii

Dén-2 (PR-159) human challenge: virus

D. Container Test Freeze Dried Final Product

- 1) Date of Freeze-drying: January 1981
- 2) Infectivity Titer: (11 vials individually titrated) 4.05×10^3 pfu/ml
- 3) Identity: Breakthrough neutralization test performed with D2 immune ascites (WRAIR WR-D2-3H5) fluid prepared from hybridoma in mice at T3M.
- 4) Microbial Sterility: Candidate dengue virus. Freeze-dried final product.

<u>Test Volume</u>	<u>Temp</u>	<u>Media</u>	<u>Inoc. size/ Culture</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
11 mi	Room	TSB (BBL, Cockeysville, Md)	1 ml/40 ml	7 Feb 83	21 Feb 83	Satisfactory	Book 45 pp 2-5
11 mi	31°C	Thio.(BBL, Cockeysville, Md)	1 ml/40 ml	7 Feb 83	21 Feb 83	Satisfactory	Book 45 pp 2-5
5)	<u>General Safety Test on Freeze Dried Final Product</u>						

<u>Animal</u>	<u>No.</u>	<u>Dose</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
Guinea Pig, male, Hartley, 200-250 g (Simonsen Labs, Gilroy, Calif.)	2	5 ml i.p.	19 Apr 83	31 May 83	Satisfactory	Book 44 pp 35-37
Adult mice, female, Swiss Webster, 17-20 g (Simonsen Labs, Gilroy, Calif.)	2	0.5 ml i.p.	21 Jan 83	11 Feb 83	Satisfactory	Book 44 pp 14-15

Den-2 (PR-159) human challenge virus

Certifications:

Approved:

 James T. Donagh P.D. Quality Assurance Officer

11/29/83 Date

Approved:

 M. Hauck

Project Director

12 Jan. 1984 Date

Department of Tropical Medicine & Medical Microbiology
 John A. Burns School of Medicine
 University of Hawaii

FINAL REPORT

Production Seed, June 1989

A. Sterility Test*

1) Microbial Sterility: Pre-Inoculation fluid

<u>Test Volume</u>	<u>Temp</u>	<u>Media</u>	<u>Inoc. size/Culture</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
5 ml	Room	TSB (BBL, Cokessville, Md.)	1 ml/40 ml	2 Feb 84	16 Feb 84	Satisfactory	Book 45, pp. 69-75
5 ml	31°C	Thio. (BBL, Cokessville, Md.)	1 ml/40 ml	2 Feb 84	16 Feb 84	Satisfactory	Book 45, pp. 69-75

2) Microbial Sterility: Control fluid

<u>Test Volume</u>	<u>Temp</u>	<u>Media</u>	<u>Inoc. size/Culture</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
10 ml	Room	TSB (BBL, Cokessville, Md.)	1 ml/40 ml	2 Feb 84	16 Feb 84	Satisfactory	Book 45, pp. 69-75
10 ml	31°C	Thio. (BBL, Cokessville, Md.)	1 ml/40 ml	2 Feb 84	16 Feb 84	Satisfactory	Book 45, pp. 69-75
6 ml		Mycoplasma broth and Agar	1 ml/tube x 4 tubes	2 Feb 84	3 Mar 84	Satisfactory	Book 45, pp. 69-75

3) Microbial Sterility: Candidate Dengue Virus, Unclarified Fluid Pool

<u>Test Volume</u>	<u>Temp</u>	<u>Media</u>	<u>Inoc. size/Culture</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
10 ml	Room	TSB (BBL, Cokessville, Md.)	1 ml/40 ml	2 Feb 84	16 Feb 84	Satisfactory	Book 45, pp. 69-75
10 ml	31°C	Thio. (BBL, Cokessville, Md.)	1 ml/40 ml	2 Feb 84	16 Feb 84	Satisfactory	Book 45, pp. 69-75
6 ml		Mycoplasma broth and Agar	1 ml/tube x 4 tubes	2 Feb 84	3 Mar 84	Satisfactory	Book 45, pp. 69-75

* Media will show no growth at 18-24 hours incubation for sterility.

TSB: Will support growth of Bacteroides vulgaris ATCC 8482 and Micrococcus luteus ATCC 12-9341.

THIO: Will support growth of Micrococcus luteus ATCC 12-9341 and Candida albicans ATCC E-10231.

Mycoplasma media: Will support growth of M. pneumoniae ATCC 15511 and M. hominis ATCC 23114.

DEN-3 Production Seed

B. Cell Culture Safety Test for Candidate Challenge Virus, Fluid Pool

Pre-inoculation fluid: Inoculation size - 3.5 ml/25 cm² flask

<u>Cells</u>	<u>No. of Culture</u>	<u>Volume</u>	<u>Tested</u>	<u>Days Observed*</u>	<u>Results</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Int'l. location</u>
Primary Green monkey kidney Source - T3M Lot # 1X	3 flasks	10 ml	21	Satisfactory	24 Feb 84	16 Mar 84	Book 44, pp. 106-111	
Subculture PGK cell Source - T3M Lot # IX	3 flasks	10 ml	21	Satisfactory	19 Mar 84	9 Apr 84	Book 44, pp. 119-120	
Primary Rhesus monkey kidney Source - Flow Lot #59272	3 flasks	10 ml	21	Satisfactory	24 Feb 84	16 Mar 84	Book 44, pp. 106-111	
Primary rabbit kidney Source - Flow Lot #59297	3 flasks	10 ml	21	Satisfactory	24 Feb 84	16 Mar 84	Book 44, pp. 106-111	
WI-38 cells Passage - 35 Source - T3M	3 flasks	10 ml	21	Satisfactory	24 Feb 84	16 Mar 84	Book 44, pp. 106-111	

* FDA regulations require a minimum of 14 days observation of cell cultures

DEN-3 Production Seed

B. Cell Cultures (Continued)

Candidate Dengue Virus, Fluid Pool

- 1) Infectivity Titer: (Mean of 3 determinations) 3.8×10^5 pfu/ml
- 2) Safety Test: Neut virus inoc: 1.7 ml per 25 cm² flask. Control fluid inoc: 3.5 ml per 25 cm² flask.
Unneut virus inoc: 3.5 ml per 25 cm² flask.

Cell	Neut Virus Volume	No. of Cultures	Con. Fluids Volume	Unneut D ₃		No. of Cultures	Date Initiated	Date Completed	Results	Date Location
				Incubate 39°C Volume	No. of flasks					
Primary green monkey kidney Source - T3M Lot # IX	5 ml	3 flasks	10 ml	10 ml	3 flasks	10 ml	24 Feb 84	16 Mar 84	Satisfactory	Book 44, pp. 106-111
Subculture PGK Source - T3M Lot # IX	10 ml	3 flasks	10 ml	10 ml	3 flasks	10 ml	24 Feb 84	16 Mar 84	Satisfactory	Book 44, pp. 119-120
Primary Rhesus monkey kidney Source - Flow Lot #59272	5 ml	3 flasks	10 ml	10 ml	3 flasks	10 ml	24 Feb 84	16 Mar 84	Satisfactory	Book 44, pp. 106-111
Primary rabbit kidney Source - Flow Lot #59297	5 ml	3 flasks	10 ml	10 ml	3 flasks	10 ml	24 Feb 84	16 Mar 84	Satisfactory	Book 44, pp. 106-111
WI-38, passage 5 Source - T3M Lot # p35	5 ml	3 flasks	10 ml	10 ml	3 flasks	10 ml	24 Feb 84	16 Mar 84	Satisfactory	Book 44, pp. 106-111

DEN-3 Production Seed Virus

C. Animal inoculation

Animal	Number Inoculated				Date Initiated	Date Completed	Results	Data location
	Neut Virus	Unneut Virus	Con. Fluid					
Adult mice, female, Swiss Webster 17-20 g* 0.03 ml i.c. + 0.5 ml i.p.	---	20	---		6 Feb 84	2 Apr 84	Satisfactory	Book 44, pp. 99-100 115-118
Suckling mice, Swiss Webster 1 day* 0.01 ml i.c. + 0.1 ml i.p.	8 litters	--	3 litters	6 Feb 84	15 May 84	Satisfactory	Book 44, pp. 94-98 115-118 123-125	
Subpassage (suckling mice) 0.01 ml i.c. + 0.1 ml i.p.	4 litters	--	---	12 Mar 84	26 Mar 84	Satisfactory	Book 44, pp. 112-113	
Guinea pig, male, Hartley 200-250 g* 0.1 ml i.c. + 5 ml i.p.	---	2	2	6 Feb 84 28 Mar 84	19 Mar 84 9 May 84	Satisfactory	Book 44, pp. 101-103 121-122	
Rabbit, male, New Zealand 1.4-1.8 kg 9 ml s.c. + 0.25 ml x 4 i.d.	---	2	2	6 Feb 84	27 Feb 84	Satisfactory	Book 44, pp. 101-105	
i.c. = intracerebral i.p. = intraperitoneal	s.c. = subcutaneous	i.d. = intradermal						

Procedure for identification:

Mouse Safety Test: Each individual cage is labeled with the number, virus lot number and date of test.

Guinea Pig Safety Test: Each cage is labeled with the serial number, virus lot number and date of test.

Rabbit Safety Test: Rabbit ears are tattooed with an individual serial number. Cage is labeled to show virus lot number and date of test.

* Source of mice and guinea pigs: Simonsen Labs, Gilroy, California

Source of rabbits: Smitty's Farm, Kaneohe, Hawaii

DEN-3 Production Seed Virus

D. Container Test Freeze Dried Final Product

1) <u>Date of Freeze-drying:</u>				
2) <u>Infectivity Titer:</u> (20 vials individually titrated)	Mean = 7.7×10^5 pfu/ml (Range 3.0×10^5 - 1.3×10^6 pfu/ml)			
3) <u>Identity:</u> Breakthrough neutralization test performed with D ₁ mouse hyperimmune ascitic fluid prepared at WRAIR	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
19 Jun 73	1 Mar 84		In Progress	
4) <u>Microbial Sterility:</u> Candidate dengue virus. Freeze dry final product.	<u>Test Volume</u>	<u>Temp</u>	<u>Media</u>	<u>Inoc. size/Culture</u>
20 ml	Room	TSB (BBL, Cockeysville, Md.)	1 ml/40 ml bottle	2 Feb 84
20 ml	31°C	Thio. (BBL, Cockeysville, Md.)	1 ml/40 ml bottle	2 Feb 84
				16 Feb 84
				Satisfactory
				Book 45, pp. 69-75
5) <u>General Safety Test on Freeze Dried Final Product</u>	<u>Animal</u>	<u>No.</u>	<u>Dose</u>	<u>Date Initiated</u>
				<u>Date Completed</u>
Guinea pig, male, Hartley, 200-250 g (Simonsen Labs, Gilroy, California)	2	5 ml i.p.	28 Mar 84	9 May 84
Adult mice, female, Swiss Webster, 17-20 g (Simonsen Labs, Gilroy, California)	2	0.5 ml i.p.	6 Feb 84	27 Feb 84
				Satisfactory
				Book 44, pp. 99-100
				Book 44, pp. 121-122

Candidate: Dengue Virus

Certifications:	Approved:	Quality Assurance Officer
	<u>John D. Smith</u>	
		7-19-84
		Date
Approved:	Project Director	
	<u>Dr. J. M. Macalit</u>	
		7-19-84
		Date

Department of Tropical Medicine & Medical Microbiology
 John A. Burns School of Medicine
 University of Hawaii

FINAL REPORT

Test item: Dengue-4 (H-241) parent, non-attenuated, Dec 82, Lot no. 1 (human challenge virus)

A. Sterility Test*

1) Microbial Sterility: Pre-Inoculation fluid

<u>Test Volume</u>	<u>Temp</u>	<u>Media</u>	<u>Inoc. size/ Culture</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
5 ml	Room	TSB (BBL, Cokeysville, Md)	1 ml/40 ml	1 July 83	15 July 83	Satisfactory	Book 45 pp 13-16
5 ml	31°C	Thio. (BBL, Cokeysville, Md)	1 ml/40 ml	1 July 83	15 July 83	Satisfactory	Book 45 pp 13-16

2) Microbial Sterility: Control fluid

<u>Test Volume</u>	<u>Temp</u>	<u>Media</u>	<u>Inoc. size/ Culture</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
10 ml	Room	TSB (BBL, Cokeysville, Md)	1 ml/40 ml	1 July 83	15 July 83	Satisfactory	Book 45 pp 13-16
10 ml	31°C	Thio. (BBL, Cokeysville, Md)	1 ml/40 ml	1 July 83	15 July 83	Satisfactory	Book 45 pp 13-16
6 ml		Mycoplasma broth and Agar 1 ml/tube x 4 tubes 0.2 ml/plate x 10 plates (BBL, Cokeysville, Md)		1 July 83	29 July 83	Satisfactory	Book 45 pp 13-16

3) Microbial Sterility: Candidate Dengue Virus, Unclarified Fluid Pool

<u>Test Volume</u>	<u>Temp</u>	<u>Media</u>	<u>Inoc. size/ Culture</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
10 ml	Room	TSB (BBL, Cokeysville, Md)	1 ml/40 ml	11 Apr 83	25 Apr 83	Satisfactory	Book 45 pp 7-12
10 ml	31°C	Thio. (BBL, Cokeysville, Md)	1 ml/40 ml	11 Apr 83	25 Apr 83	Satisfactory	Book 45 pp 7-12
6 ml		Mycoplasma broth and Agar 1 ml/tube x 4 tubes 0.2 ml/plate x 10 plates (BBL, Cokeysville, Md)		11 Apr 83	16 May 83	Satisfactory	Book 45 pp 7-12

* Media will show no growth at 18-24 hours incubation for sterility.

TSB: Will support growth of Bacteroides vulgaris ATCC 8482 and Micrococcus lutea ATCC 12-9341.

THIO: Will support growth of Micrococcus lutea ATCC 12-9341 and Candida albicans ATCC E-10231.

Mycoplasma media: Will support growth of M. pneumoniae ATCC 15531 and M. arthritidis.

Den-4 (H-241) human challenge virus

B. Cell Culture Safety Test for Candidate Challenge Virus, Fluid Pool

<u>Pre-inoculation fluid:</u>	Inoculation size--3.5 ml/25 cm ² flask				<u>Date Initiated</u>	<u>Date Completed</u>	<u>Data Location</u>
<u>Cells</u>	<u>No. of Culture</u>	<u>Volume Tested</u>	<u>Days Observed</u>	<u>Results</u>			
Primary green monkey kidney. Source--T3M Lot # VIII	3 flasks	10 ml	14	Satisfactory	22 Jul 83	5 Aug 83	Book 44 pp 79-80
Subculture PGMK cell. Source--T3M Lot # VII	3 flasks	10 ml	14	Satisfactory	5 Aug 83	18 Aug 83	Book 44 pp 85-86
Primary Rhesus monkey kidney. Source-- Flow Lot # 57083	3 flasks	10 ml	14	Satisfactory	11 Jul 83	25 Jul 83	Book 44 pp 85-86
Primary rabbit kidney. Source--Flow Lot # 56597	3 flasks	10 ml	14	Satisfactory	11 Jul 83	25 Jul 83	Book 44 pp 75-77
WI-38 cells, Passage--22 Source--T3M	3 flasks	10 ml	14	Satisfactory	11 Jul 83	25 Jul 83	Book 44 pp 75-77

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B. Cell Cultures (cont.)

Candidate Dengue Virus, Fluid Pool

- 1) Infectivity Titer: (Mean of 3 determinations) 4 weeks no titer (leakage of CO₂ into vials in improperly sealed can caused pH change which is suspected of killing virus)
- 2) Safety Test: Neut. virus inoc: 1.7 ml per 25 cm² flask. Control fluid inoc.: 3.5 ml per 25 cm² flask; Unneut. virus inoc.: 3.5 ml per 25 cm² flask

<u>Cell</u>	<u>Neut. Virus Vol.</u>	<u>No. of Culture</u>	<u>Con. Fluids Vol.</u>	<u>No. of Culture</u>	<u>Days Observed</u>	<u>Date Initiated</u>	<u>Date Completed</u>	<u>Results</u>	<u>Data Location</u>
Primary Green monkey kidney Source--T3M Lot # VIII	5 ml	3 flasks	10 ml	3 flasks	14	27 May 83 22 Jul 83	10 Jun 83 5 Aug 83	Satisfactory	Book 44 pp 59-61 Book 44 pp 79-80
Subculture PGMR Source-T3M Lot # VII	10 ml	3 flasks	10 ml	3 flasks	14	5 Aug 83	18 Aug 83	Satisfactory	Book 44 pp 85-86
Primary Rhesus monkey kidney Source--Flow Lot # 56555 57083	5 ml	3 flasks	10 ml	3 flasks	14	13 May 83 11 Jul 83	27 May 83 25 Jul 83	Satisfactory	Book 44 pp 53-54 Book 44 pp 75-77
Primary rabbit kidney Source--Flow Lot # 57116 56597	5 ml	3 flasks	10 ml	3 flasks	14	11 May 83 11 Jul 83	25 May 83 25 Jul 83	Satisfactory	Book 44 pp 50-52 Book 44 pp 75-77
WI-38, passage Source-T3M Lot # 30, 22	5 ml	3 flasks	10 ml	3 flasks	14	19 May 83 11 Jul 83	2 Jun 83 25 Jul 83	Satisfactory	Book 44 pp 55-56 Book 44 pp 75-77

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2. Animal inoculation

Animal	No. inoculated			Date Initiated	Date Completed	Results	Location
	Neut. Virus	Unneut. Virus	Con. Fluid				
Adult mice, female, Swiss Webster, 17-20 g* 0.03 ml i.c. + 0.5 ml i.p.	---	20	---	24 May 83	14 Jun 83	Satisfactory	Book 44 pp 57-59
Suckling mice, Swiss Webster, 1 day* 0.01 ml i.c. + 0.1 ml i.p.	8 litters	--	3 litters	29 Apr 83	27 Jun 83	Satisfactory	Book 44 pp 45-49, 66-69
Subpassage (suckling mice) 0.01 ml i.c. + 0.1 ml i.p.	3 litters	--	---	22 Sep 83	23 Oct 83		Book 44 pp 87-89
Guinea pig, male, Hartley, 200-250 g* 0.1 ml i.c. + 5 ml i.p.	---	2	2	3 Jun 83	17 Jun 83	Satisfactory	Book 44 pp 63-65
Rabbit, male, New Zealand, 1.4-1.8 kg* 9 ml s.c. + 0.25 ml x 4 i.d.	---	2	2	19 Apr 83 28 Jul 83	31 May 83 8 Sep 83	Satisfactory	Book 44 pp 35-37 Book 44 pp 81-82

i.c. = intracerebral

i.p. = intraperitoneal

s.c. = subcutaneous

i.d. = intradermal

Procedure for identification:

Mouse Safety Test: Each individual cage is labeled with the number, virus lot number and date of test.

Guinea Pig Safety Test: Guinea pig ears are tattooed with individual serial number. Each cage is labeled with the serial number, virus lot number and date of test.

Rabbit Safety Test: Rabbit ears are tattooed with an individual serial number. Cage is labeled to show virus lot number and date of test.

* Source of mice and guinea pigs: Simonson Labs, Gilroy, California; Sasco Labs, Omaha Nebraska.
Source of rabbits: Smitty's Farm, Kaneohe, Hawaii.

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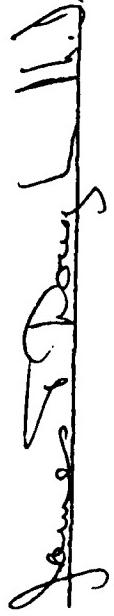
D. Container Test Freeze Dried Final Product

1) Date of Freeze-drying:	December 1982		
2) Infectivity Titer:	(12 vials individually titrated) 4.41×10^3 pfu/ml		
3) Identity:	Breakthrough neutralization test performed with D4 immune ascites fluid prepared in mice by WRAIR.		
Test Volume	Temp	Inoc. size/ Culture	Date Completed
12 ml	Room	TSB (BBL, Cokeysville, Md)	20 Sep 83
12 ml	31°C	Thio.(BBL, Cokeysville, Md)	20 Sep 83
4) Microbial Sterility: Candidate dengue virus. Freeze-dried final product.			
Test Volume	Temp	Inoc. size/ Culture	Date Completed
12 ml	Room	TSB (BBL, Cokeysville, Md)	27 Jul 83
12 ml	31°C	Thio.(BBL, Cokeysville, Md)	29 Jul 83
5) General Safety Test on Freeze Dried Final Product			
Animal	No.	Dose	Date Completed
Guinea pig, male, Hartley, 200-250 g (Simonsen Labs, Gilroy, Calif)	2	5 ml i.p.	28 Jul 83
Adult mice, female, Swiss Webster, 17-20 g (Simonsen Labs, Gilroy, Calif)	2	0.5 ml i.p.	8 Sep 83
6) Assay Test			
Test	Initiated	Completed	Results
Neutralization test	20 Sep 83	10 Nov 83	Satisfactory
Identity of virus is Dengue 4			Book 46 pp
Test	Date Initiated	Date Completed	Data Location
Neutralization test	20 Sep 83	10 Nov 83	Book 45 pp 16-18
Identity of virus is Dengue 4			Book 45 pp 81-82
Neutralization test	20 Sep 83	12 Aug 83	Satisfactory
Identity of virus is Dengue 4			Book 45 pp 16-18
Neutralization test	20 Sep 83	12 Aug 83	Satisfactory
Identity of virus is Dengue 4			Book 44 pp 81-82
Neutralization test	20 Sep 83	18 Aug 83	Satisfactory
Identity of virus is Dengue 4			Book 44 p 83

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Certifications:

Approved:

 James D. Donnelly, M.D.

Quality Assurance Officer

11/29/83 Date

Approved:

 John J. Marshall

Project Director

17 Jan. 1984 Date

END

DATE

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